SUPPLEMENTARY TABLES S1, S2 AND S3

<u>Supplementary Table S1</u> Plasmids constructed for this work.

	1 (0000) 11 1 : 1 : 1 : 1
	II. (2002) Nucleic Acids Res., 30 , 1-
reporter 11.	, , ,
transcribed by	
RNA Pol I	
pHD 1343 CAT-GC-EP1 Irmer, H. and C	layton, C.E. (2001) <i>Nucleic Acids</i>
reporter Res., 29, 4707-	4715.
transcribed by T7	
RNA polymerase	
	s amplified with the forward primer 5'-
	ttttgatcttgtatcttc-3' and with the reverse
	gattatacgctgccaatacccagtgcag-3',
	II and Xbal into the Bla/V5 vector
	n part (accession number
	was amplified with the forward primer
	gttccgtggaaggttcgaggta-3' and the
	-atatgggcccaatacatgcactgcgcaccgcc-3',
	I and XhoI into the Bla/V5 vector
pHD 1843 CAF1 RNAi Part of the	CAF1 ORF (accession number
	vas amplified using forward primer 5'-
	acagaggatgtg-3' and reverse primer 5'-
	atcttcacagagcc-3', PCR product was
cloned into p2T7	ODE (accesion number
	ORF (accession number as amplified using forward primer 5'-
	caatatcgtca-3' and reverse primer 5'-
cloped into n2T7	aatcaagcgatct-3', PCR product was
	ORF was amplified using forward
'	atctgcatgccgtcgttatcgtgacccttt-3' and
	-cggaattcgtcgactggtgcgagtttgtctcttg-3'.
	as cloned with Sall and Spel into
	hat the second insert was cloned with
	nto PAN2 insert1/pHD 1144. The PAN2
	ut of PAN2 insert1/ PAN2 insert2/1144
	BgIII. pHD 1145 was linearised with
	II and the PAN2 stuffer was inserted.
	Tb927.5.1270 gene was amplified
1, 1	TGCATGCCCACAGGAGGAGGTAACCAA
	GTCGACcatcatcatcatc then
cloned to giv	re a stem-loop into pHD1145
(1907 is in pHD1)	
	Tb11.22.0004 gene was amplified
	TGCATGCTGTTAAACCCACTGGAAGGC
1 ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	CTGCAGAACACACGAGTGAAGTTGCG
then cloned t	to give a stem-loop into
pHD1145 (1908 i	= -

Supplementary Table S2

Cloned 5' and 3' ends from circularised *RPL37A* mRNA (encoded by locus Tb10.100.0110). "Position on SL" denotes the furthest upstream *SL* nucleotide: A full-length capped mRNA stops at nt 0 and one with only one nt of the *SL* would stop at nt 39. "Nt before ATG" is the length of the 5' UTR plus *SL*. The full-length clones were obtained from the 300nt band. For sequences see Supplementary Figure S4. "TAP decap" indicates removal of the cap with tobacco acid pyrophosphatase. This digestion neither reduced the number of PCR cycles required to obtain detectable products, nor influenced the structures of the products obtained. We suspect that this is because the four methylated nucleotides proximal to the cap at the 5'-end of the trypanosome spliced leader inhibit reverse transcriptase {Freistadt, 1988 #1748;Freistadt, 1987 #1749;Perry, 1987 #1750;McNally, 1992 #691;Ullu, 1991 #907}. To solve this problem, we removed the 5'-end of the spliced leader using a specific oligonucleotide and RNase H. Lanes denoted "N" were made using a nested PCR, the others with a single primer pair. If two lanes have the same clone number (a and b) then the clone included an open reading frame, with different amplified ends at the extremities (3'-UTR - 5'-UTR - ORF - 3'-UTR - 5'-UTR). This could happen if reverse transcriptase had read right around the circle, then incomplete PCR products originating from different mRNAs hybridised within the ORF during the PCR reaction.

#In these case sequencing was only possible from one end, and terminated in the poly(A), so the number of A's present may be greater than indicated. It is also possible that the oligos were deleted during plasmid replication.

Although a few non-A residues were found in the poly(A) tails, these were no more frequent than nucleotide changes within the rest of the sequences so were probably PCR artefacts (Supplementary Figure S4).

Clone*	TAP decap	nt of SL	Nt before ATG	Nt after stop codon	Poly A Tail length
Full length	<u> </u>	39	55	(no Poly A) 108-159	lengin
XRNA+, F		39	33	100-139	
R-3-1	_	24	41	127	31
R-3-2	_	21	38	136	35
R-3-3	_	26	43	108	36
R-3-4	_	18	35	136	37
R-3-5	_	18	35	108	42
XRNA+		10	00	100	74
4-4Na	+	7	24	108	32
4-6	+	7	24	108	37
3-1a	_	24	41	108	31
3-1N	_	8	25	108	5
3-110	_	8	25	108	48
3-2N	-	8	25	108	41
3-3a			13		41
	-	-		108	62
3-3N	-	8 7	25	136	
3-4a	-		24	108	50
3-5	-	7	24	108	93
4-1Na	+	8	25	47	-
4-1Nb	+	8	25	24	-
4-2Na	+	11	28	83	-
4-2Nb	+	34	51	65	
4-3	+	8	25	91	-
4-3N	+	29	46	10	-
4-4Nb	+	8	25	83	-
4-5Na	+	11	28	83	-
4-5Nb	+	7	24	11	-
3-4Na	-	8	25	30	-
3-4Nb	-	10	27	6	-
3-5Na	-	8	25	140	-
3-5Nb	-	8	25	23	-
XRNA RN	lAi, RNas		T	1	
R-1-1	-	26	43	108	33
R-1-2	-	24	41	136	33
R-1-3	-	26	43	136	32
R-1-4	-	18	35	136	43
R-1-5	-	18	35	108	39
XRNA RN	<u>lAi</u>	T	T		
1-2N	-	-	4	136	26
1-3	-	7	24	108	32
1-3Na	-	-	6	108	5
1-3Nb	-	-	-7	108	39
1-5N	-	?		131	35#
1-7	-	-	1	109	10
2-1Na	+		10	136	22
2-2a	+	7	24	108	34
2-3a	+	7	24	159	112
2-5Nb	+	-	6	108	29
1-1N	-	9	26	-	-
1-4Na	-	29	46	85	-
1-4Nb	-	11	28	144	-
2-1Nb	+	20	37	70	-
2-2Na	+	9	26	42	-
2-2Nb	+	-	0	29	-
2-3Na	+	20	37	35	-
2-3Nb	+	11	28	97	-
2-4a	+	24	41	63	-
2-5Na	+	8	25	71	-

<u>Supplementary Table S3</u>
Cloned 5' and 3' ends from circularised *EP* mRNA. Clones from all different *EP* loci are included. Clones designated A and B are from two independent experiments. For experiment B, "a" denotes clones from nested PCR "a" (Figure 7C) and "b" from PCR "b". "Sequence no" refers to the number of the sequence in Figure S6.

For A and B, the first PCR was done with primers RT and 31 and the second with primers 52 and 32 " (Figure 7C). The destabilising 26mer is at nt 134-159 of the 3'-UTR, so is present in 3' fragments of 159 nt and above. *** primary transcript, retains the transcription start site.

Full-length No decap St. ATG stop Pull-length No decap St. ATG	Clone	Coguence	TAP	nt of	nt 5' to	nt 3' to	n/A)
Full-length 39	Cione	Sequence					p(A)
Name	Full longth	110	uecap				
Bb-3-2 EP-3-2 -		!!		39	70	297	
Ba-3-1			1		50	4-	10
Ba-3-2 pEP-3-2 - 25 57 57 11 Ba-3-3 pEP-3-3 - - -13 22 4 Bb-3-4 EP-3-4 - 29 61 -4 6 Bb-3-5 EP-3-5 - 24 56 38 - Bb-3-5 EP-3-5 - 26 58 39 - XRNA+, no RNase H Ba-4-1 pEP-4-1 - *** 115 40 4 Ba-4-2 pEP-4-2 - *** 115 63 3 A-3-2 EP1-3-2 - 26 58 35 11 A-3-4 EP1-3-4 - 25 57 13 12 A-3-4 EP1-3-6 - 34 66 3 17 A-4-2 EP1-4-2 + 26 58 105 17 A-4-3 EP1-4-3 + 26 58 105 17 A-4-3 EP1-4-3 + 26 58 105 17 A-4-4 EP1-4-1 - 26 58 4 3 Bb-4-1 EP-4-1 - 26 58 4 3 Bb-4-2 EP4-2 - 9 41 43 7 Bb-4-3 EP4-3 - 25 57 36 12 Bb-4-5 EP4-5 - 20 17 28 Ba-4-6 pEP-4-5 - 20 17 28 Ba-4-6 pEP1-3 - - 46 55 - A-3-3 EP1-3-3 - - 46 55 - A-3-5 EP1-3-5 - 27 59 -10 - A-4-6 EP1-4-6 + - - 17 16 - A-4-7 EP1-4-1 + - 17 16 - A-4-8 EP1-4-1 - 26 58 16 10 Bb-4-1 EP1-4-1 + - 17 16 - A-4-1 EP1-4-1 + - 27 59 -1 - Ba-4-3 pEP-3 - 27 59 -1 - Ba-4-4 EP1-4-4 - 28 60 1 - A-4-5 EP1-4-5 - 20 62 4 - XRNA RNAI, RNase H Bb-1-4 EP1-1 - 26 58 4 - Ba-1-1 pEP1-1 - 26 58 -1 - Ba-1-2 pEP1-2 - 20 62 4 - A-1-1 EP1-3 - 24 56 55 - A-1-2 EP1-1-3 - 24 56 55 - A-1-3 EP1-3 - 27 59 4 - Ba-1-1 pEP1-1 - 26 58 -1 - Ba-1-2 pEP1-2 - 20 28 - A-1-5 EP1-3 - 27 59 4 - Ba-1-6 EP1-1 - 26 58 -1 - Ba-1-7 pEP1-1 - 26 58 -1 - Ba-1-8 pEP1-1 - 26 58 -1 - Ba-1-9 pEP1-1 - 26 58 -1 - Ba-1-1 pEP1-1 - 26 58			-				
Ba-3-3			-				
Ba-3-4		•	-	25			
Bb-3-4 EP-3-4 - 24 56 -5 - 8 Bb-3-5 EP-3-5 - 24 56 38 - 8 Ba-3-5 EP-3-5 - 26 58 39 - 2	Ba-3-3	pEP-3-3	-	-	-13	22	
Bb-3-5	Ba-3-4	pEP-3-4	-	29	61	-4	6
Bb-3-5	Bb-3-4	EP-3-4	-	24	56	-5	-
Ba-3-5 DEP-3-5 - 26 58 39 -	Bb-3-5	EP-3-5	-	24	56	38	-
XRNA+, no RNase H Ba-4-1	Ba-3-5	pEP-3-5	-	26	58	39	-
Ba-4-1 pEP-4-1 - *** 115 40 4	XRNA+. no			I.			
Ba-4-2			_	***	115	40	4
A-3-2 EP1-3-2 - 26 58 35 11 A-3-4 EP1-3-4 - 25 57 13 12 A-3-6 EP1-3-6 - 34 66 3 17 A-4-2 EP1-4-2 + 26 58 105 17 A-4-3 EP1-4-3 + 26 58 -14 5 A-4-6 EP1-4-6 + - -13 53 4 Bb-4-1 EP4-1 - 26 58 -14 5 Bb-4-2 EP4-1 - 26 58 4 3 7 Bb-4-3 EP4-2 - 9 41 43 7 7 28 8 4 3 7 8 12 8 4 3 7 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12 8 12				***			
A-3-4				26			
A-3-6							
A-4-2 EP1-4-2 + 26 58 105 17 A-4-3 EP1-4-3 + 26 58 -14 5 A-4-6 EP1-4-6 +13 53 4 Bb-4-1 EP-4-1 - 26 58 4 3 Bb-4-2 EP-4-2 - 9 41 43 7 Bb-4-3 EP-4-3 - 25 57 36 12 Bb-4-5 EP-4-5 20 17 28 Ba-4-5 pEP-4-5 - 29 61 36 12 A-3-1 EP1-3-1 46 55 - A-3-3 EP1-3-3 - 27 59 -10 A-4-4 EP1-4 + 28 60 1 - A-4-4 pEP-1-4 - 29 62 4 - B-4-4 pEP-1-1 pEP-1-1 pEP-1-1 pEP-1-1 pEP-1-1 pEP-1-1 pEP-1-1 pEP-1-3 pEP-1-4 pEP-1-5 pEP-1-6 pEP-1-6 pEP-1-6 pEP-1-6 pEP-1-7 pEP-1-1 pEP-1-							
A-4-3							
A-4-6							
Bb-4-1							
Bb-4-2							
Bb-4-3			-				
Bb-4-5			-				
Ba-4-5 pEP-4-5 - 29 61 36 12 A-3-1 EP1-3-1 - - -46 55 - A-3-3 EP1-3-3 - - 0 22 - A-3-5 EP1-3-5 - 27 59 -10 - A-4-1 EP1-4-1 + - 17 16 - A-4-4 EP1-4-1 + - 17 16 - A-4-5 EP1-4-5 + - -16 -14 - Bb-4-4 EP-4-4 - 8 40 122 - Ba-4-3 pEP-4-3 - 27 59 -1 - Ba-4-3 pEP-4-4 - 29 62 4 - XRNA RNAI, RNAI, RNAI RNase H - 29 62 4 - Bb-1-2 EP-1-2 - 26 58 16 10 Bb-1-2 EP			-	25	57		
A-3-1	Bb-4-5	EP-4-5	-	-	20	17	28
A-3-3	Ba-4-5	pEP-4-5	-	29	61	36	12
A-3-5	A-3-1	EP1-3-1	-	-	-46	55	-
A-4-1 EP1-4-1 + - 17 16 - A-4-4 EP1-4-4 + 28 60 1 - A-4-5 EP1-4-5 + - -16 -14 - Bb-4-4 EP-4-4 - 8 40 122 - Ba-4-3 pEP-4-3 - 27 59 -1 - Ba-4-4 pEP-4-4 - 29 62 4 - XRNA RNAI, RNASE H Bb-1-3 EP-1-4 - 26 58 16 10 Bb-1-2 EP-1-2 - 23 55 19 - Bb-1-3 EP-1-3 - 26 58 -12 - Ba-1-1 pEP-1-1 - 26 58 -12 - Ba-1-2 pEP-1-2 - 26 58 31 - Ba-1-3 pEP-1-3 - 27 59 4 - Ba-1-4 pEP-1-4 - 26 58 -4 -	A-3-3	EP1-3-3	-	-	0	22	-
A-4-1 EP1-4-1 + - 17 16 - A-4-4 EP1-4-4 + 28 60 1 - A-4-5 EP1-4-5 + - -16 -14 - Bb-4-4 EP-4-4 - 8 40 122 - Ba-4-3 pEP-4-3 - 27 59 -1 - Ba-4-4 pEP-4-4 - 29 62 4 - XRNA RNAI, RNASE H Bb-1-3 EP-1-4 - 26 58 16 10 Bb-1-2 EP-1-2 - 23 55 19 - Bb-1-3 EP-1-3 - 26 58 -12 - Ba-1-1 pEP-1-1 - 26 58 -12 - Ba-1-2 pEP-1-2 - 26 58 31 - Ba-1-3 pEP-1-3 - 27 59 4 - Ba-1-4 pEP-1-4 - 26 58 -4 -	A-3-5	EP1-3-5	-	27	59	-10	-
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Bb-4-4							
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Ba-1-2 pEP-1-2 - 26 58 31 - Ba-1-3 pEP-1-3 - 27 59 4 - Ba-1-4 pEP-1-4 - 26 58 -4 - Ba-1-5 pEP-1-5 - 24 56 55 - XRNA RNAi, no RNase H A-1-3 EP1-1-3 - - -48 40 5 A-1-5 EP1-1-5 - - 0 -8 5 A-2-5 EP1-2-5 + - -24 0 4 A-1-2 EP1-1-2 - - 0 28 - A-1-4 EP1-1-4 - - - 4 53 - A-1-6 EP1-1-6 - - 13 14 - A-2-1 EP1-2-1 + - - 21 135 - A-2-2 EP1-2-3 + - 0			-				
Ba-1-3 pEP-1-3 - 27 59 4 - Ba-1-4 pEP-1-4 - 26 58 -4 - Ba-1-5 pEP-1-5 - 24 56 55 - XRNA RNAi, no RNase H A-1-3 EP1-1-3 - - -48 40 5 A-1-5 EP1-1-5 - - 0 -8 5 A-2-5 EP1-1-5 - - 0 -8 5 A-2-5 EP1-2-5 + - -24 0 4 A-1-2 EP1-1-2 - - 0 28 - A-1-4 EP1-1-4 - - - 4 53 - A-1-6 EP1-1-6 - - 13 14 - A-2-1 EP1-2-1 + - -31 65 - A-2-2 EP1-2-3 + - 0 24 -<	_		-				-
Ba-1-4 pEP-1-4 - 26 58 -4 - XRNA RNAi, no RNase H A-1-3 EP1-1-3 - -48 40 5 A-1-5 EP1-1-5 - 0 -8 5 A-2-5 EP1-2-5 + - -24 0 4 A-1-2 EP1-1-2 - 0 28 - A-1-4 EP1-1-4 - - -4 53 - A-1-6 EP1-1-6 - - 13 14 - A-2-1 EP1-2-1 + - -31 65 - A-2-2 EP1-2-2 + - 21 135 - A-2-3 EP1-2-3 + - 0 24 - A-2-4 EP1-2-4 + - -50 121 - Bb-2-3 EP-2-3 - - -37 31 - Ba-2-1 pEP-2-2		· ·	-			31	1
Ba-1-5 pEP-1-5 - 24 56 55 - XRNA RNAi, no RNase H A-1-3 EP1-1-3 - - -48 40 5 A-1-5 EP1-1-5 - - 0 -8 5 A-2-5 EP1-2-5 + - - 0 4 A-2-5 EP1-2-5 + - - 0 4 A-1-2 EP1-2-5 + - - 0 28 - A-1-2 EP1-1-2-5 + - - 0 28 - A-1-2 EP1-1-2-2 - - - 4 53 - A-2-1 EP1-2-1 + - - 31 65 - A-2-2 EP1-2-2 + - 21 135 - A-2-3 EP1-2-3 + - 0 24 -	Ba-1-3		-	27	59	4	-
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<u>Supplementary Table S4</u> Cloned 5' and 3' ends from circularised *EP* mRNA. Clones designated Y and Z are from independent experiments. The first PCR was done with primers RT and 32. For experiment Y, the second PCR was with primers 51 and 33, and for Z with primers 51 and 34. Note that these results are not representative because we deliberately selected clones with longer inserts for sequencing. Statistical analysis would therefore not be appropriate.

*wild-type polyadenylation site; #ends with aat or aaat instead of aaaat. \$ends with poly(T)

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